IN THE CLAIMS

Claims 1-17 (cancel)

18. (previously presented).

Modified DNA fragments of the following structure:

5'–X–O–DNA–3'–O–Z (II)

wherein

DNA represents a DNA fragment,

X is H or H_2PO_3, Z represents $-CO-Y-CR^1=CR^2R^3$

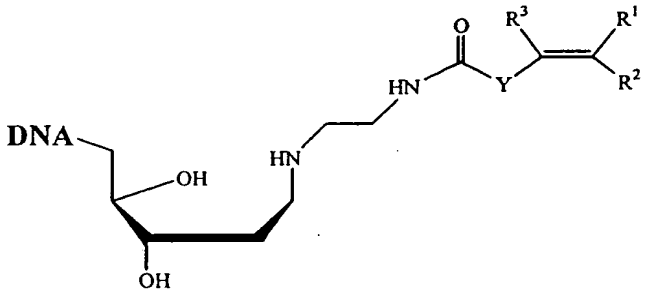
or

X is $-CO-Y-CR^1=CR^2R^3$, Z is H or H_2PO_3,

Y represents $(p\text{-}C_6H_4)_n$ where n = 0-2;

being prepared by direct acylation of DNA fragments with anhydrides of unsaturated acids;

or formula (III):



(III)

wherein:

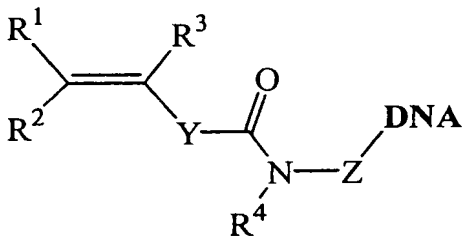
DNA represents a DNA fragment;

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$– ;

Y is $(p\text{-}C_6H_4)_n$ where n = 0-2,

being prepared by reductive amination of the purine free DNA followed by acylation of amine derivative with activated esters of unsaturated acids;

or



IV

wherein:

DNA represents a DNA fragment;

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$– ;

Y is $(p\text{-}C_6H_4)_n$ where n = 0-2;

R^4 represents H, $(CH_2)_nOH$ where n = 2-6;

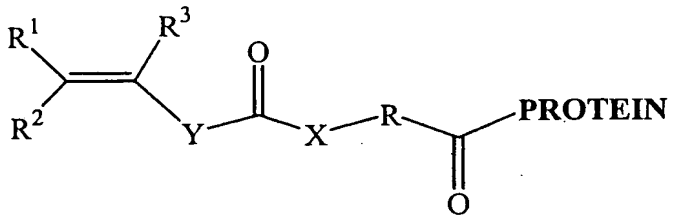
Z is $(CH_2)_nCH(CH_2OH)CH_2OX$ where n = 1-6; or $-(CH_2)_n-OX$ where n = 2-6;

X is a phosphodiester group binding an unsaturated moiety to 5'- and/or 3'- end of the DNA fragment,

being prepared by PCR-amplification using a synthetic primer bearing an unsaturated group at 5'- or 3'- end.

19. (previously presented).

Modified proteins of the following structure:



V

wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$–;

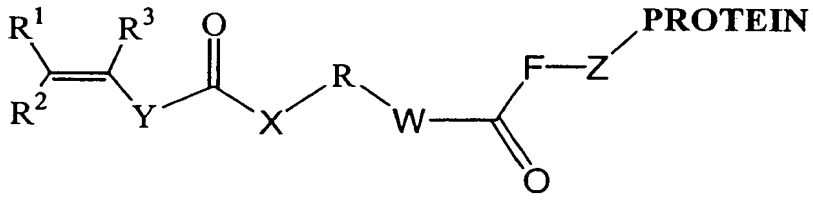
X is NH, O, CH_2, S;

Y represents $(p\text{-}C_6H_4)_n$ where n = 0-2;

R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1- 20,

being prepared by acylation of protein's free amino-groups with activated esters of unsaturated acids;

or



VI

wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$- ;

X is NH, O, S, CH_2 ;

Y is $(p\text{-}C_6H_4)_n$, where n = 0-2;

R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1-20;

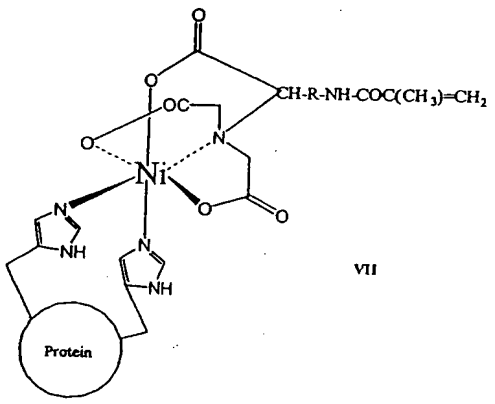
W is NH, O, CH_2;

F is $(CH_2)_n$, n=1, 2;

Z =NH, S

being prepared by alkylation of protein's amino- or sulfhydryl groups with derivatives of αβ-unsaturated and α-halocarbonyl compounds;

or



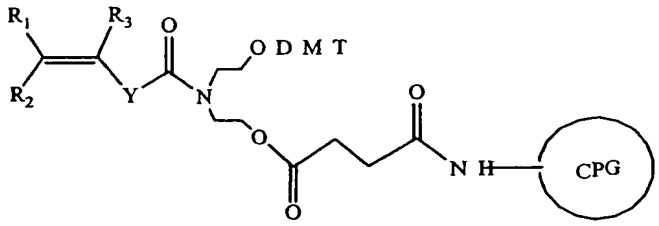
VII

wherein R represents $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1–20;

by treatment of a recombinant protein comprising an His-6 end fragment with methacrylamide derivatives of nitrilotriacetic acid in the presence of Ni(II) salts.

20. (previously presented).

A modified porous glass (CPG) of the following structure:



wherein:

R^1, R^2 are H, alkyl C_1-C_6, Ph, $PhCH_2$-;

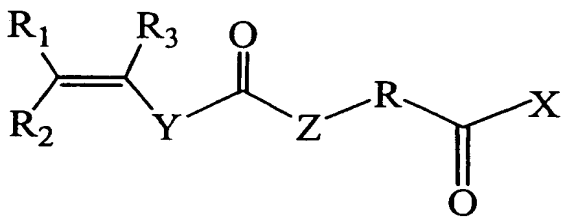
R^3 is alkyl C_1-C_6;

Y is $(p\text{-}C_6H_4)_n$, where n = 0-2,

as a carrier to insert the fragment of unsaturated acid at 3'-end of oligonucleotide of formula I according to claim 6 under conditions of automatic solid-phase synthesis.

21. (previously presented).

Activated esters of the following structure:



wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$- ;

Y is $(p\text{-}C_6H_4)_n$, where n = 0-2;

Z is NH, O, CH_2, S

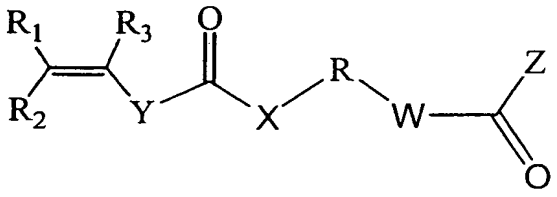
R is $(CH_2)_n$, $(CH_2CH_2O)_n$, where n = 1-20;

X is succinimidoxy-, *p*-nitrophenoxy-, pentafluoro phenoxy-, or any other readily leaving acceptor group,

as a modifying agent for preparing the protein of formula V.

22. (previously presented).

Carbonyl compounds of the following structure:



wherein

R^1, R^2, R^3 are H, alkyl C_1-C_6, Ph, $PhCH_2$- ;

Y is $(p\text{-}C_6H_4)_n$, n = 0-2;

X is NH, O, S, CH_2;

R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1-20;

W is NH, O, CH_2,

Z is a halomethyl, vinyl, or any other fragment comprising an active multiple bond,

as a modifying agent for preparing the protein of formula VI.

23. (previously presented).

Methacrylamide derivatives of nitrilotriacetic acid of general formula:

$$CH_2=C(Me)–CO-NH–R-CH(COOH)–N(CH_2COOH)_2,$$

wherein R is $(CH_2)_n$, $(CH_2CH_2O)_n$, n = 1-20,
as a modifying agent for preparing the protein of formula VII.

24. (previously presented).

Biochip accomplished based on composition of claim 1 wherein gel layer formed on a substrate is divided by empty spaces into several cells and each cell will comprise or not comprise macromolecules immobilized, and macromolecule being immobilized in various cells will have different nature and properties.

25. (previously presented).

Biochip according to claim 24 wherein said cells form the regular one- or two-dimensional structure (phase).

26. (previously presented).

Biochip according to claim 24 on preparation of which an application of the polymerization mixture on substrate is preferably carried out by using an automatic device (robot) equipped with one or more micro dispensers.

27. (previously presented).

Biochip according to claim 26 on preparation of which use is made of micro dispensers of rod type.

28. (previously presented).

Biochip according to claim 26 on preparation of which use is made of contactless micro dispensers of jet type.

29. (previously presented).

Biochip according to claim 26 on preparation of which use is made of several micro dispensers forming a regular structure.

30. (previously presented).

Biochip according to claim 24 on preparation of which one or more substrates including applied droplets of polymerization mixture, during polymerization, are placed into a sealed container under oxygen free inert atmosphere with a controlled humidity.

31 (previously presented).

Biochip according to claim 24 on preparation of which said container being filled with one of the following gases: N_2, Ar, CO_2.

32. (previously presented).

Biochip according to claim 24 on preparation of which said gaseous medium being continuously or periodically restored in the container with substrates.

33. (previously presented).

A method for performing the PCR over biochip according to claim 24 by using:

- an addition of amplification solution, forward (F) and reverse (R) primers of samples of nucleic acids under investigation,

and

- an incubation of biochip under conditions of a thermocycling treatment providing a realization of PCR-amplification.

34. (previously presented).

A method for performing the PCR over biochip according to claim 24 by using:

- an isothermal incubation of biochip with hybridization solution comprising the samples of nucleic acids under investigation to perform their hybridization with primers immobilized (synthetic oligonucleotides),
- an isothermal incubation of biochip, comprising the nucleic acids being hybridized with primers immobilized, in the amplification solution containing forward (F) and reverse (R) primers,
- replacement of the amplification solution out of biochip gel elements with hydrophobic liquid (mineral oil) which completely isolates biochip cells with each other, and
- an incubation of biochip under conditions of a thermocycling treatment providing a realization of PCR-amplification.

35. (New) A method for immobilization of biological macromolecules in hydrogels by using a composition (K) prepared by copolymerization according to the formula:

$$K=aA+bB+cC+dD+eE$$

wherein:

A is a monomer based on derivatives of acrylic and methacrylic acids;

B is a water soluble cross-linking agent;

C is a biological modified macromolecule bearing an unsaturated group;

D is a water soluble compound as a medium component for performing a copolymerization;

E is water, and

a, b, c, d, and e are percentages (X) of each ingredient in the composition, wherein for solids X=m/v x 100% and for liquids X= v/v x100%, wherein the total content of monomer and cross-linking agent ranges from 3 to 40%

$(3 \leq (a + b) \leq 40\%)$, and a monomer to cross-linking agent ratio being within a range of 97:3 to 60:40 and percentages of C, D, and E ingredients being within a range of $0.0001\% \leq c \leq 10\%$; $0\% \leq d \leq 90\%$; $5\% \leq e \leq 95\%$.

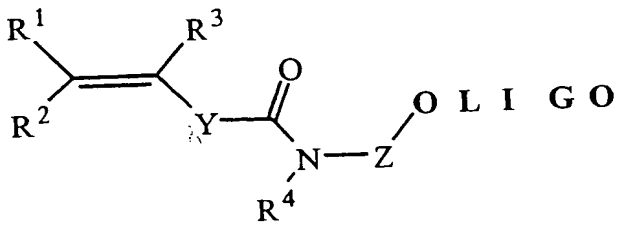
36. (New) The method of claim 35, wherein monomer A is one or more of acrylamide, methacrylamide, N-[tris(hydroxymethyl)methyl]acrylamide, and 2-hydroxyethylmethacrylate.

37. (New) The method of claim 36 wherein monomers are used separately or as a mixture.

38. (New) The method of claim 35 wherein the cross-linking agent B is one or more of N,N'-methylenbisacrylamide, N,N'-ethylenbismethacrylamide, N,N'-(1,2-dihydroxyethylene)bisacrylamide, and polyethylene glycol diacrylate.

39. (New) The method of claim 38 wherein cross-linking agents are used separately or as a mixture.

40. (New) The method of claim 35 wherein the modified biological macromolecule is of formula (I):



(I)

wherein OLIGO represents an oligonucleotide;

R^1, R^2, and R^3 are the same or different and are selected from H, alkyl C_1-C_6, Ph, $PhCH_2$-; Z is $(CH_2)_nCH(CH_2OH)CH_2OX$ where n=1-6; or $(CH_2)_n$-OX where n=2-6;

X is a phosphodiester group binding an unsaturated moiety to 5'- ,3'-end or both of the oligonucleotide;

R_4 represents H, $(CH_2)_nOH$ where n=2-6; and

Y is $(p\text{-}C_6H_4)_n$ where n=0-2.

41. (New) The method of claim 35, wherein the modified biological macromolecule is of formula (II)

5'–X–O–DNA–3'–O–Z (II)

5'-X-O-DNA-3'-O-Z (II)

wherein DNA represents a DNA fragment;

X is H or H_2PO_3; and

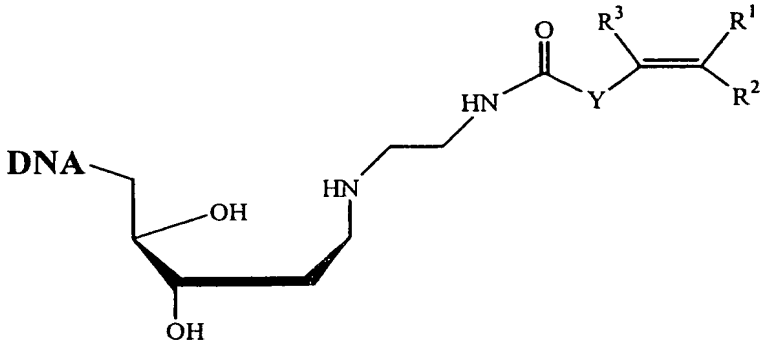
Z represents -CO-Y—CR^1=CR^2R^3 or

X is -CO-Y-CR^1=CR^2R^3, and Z is H or H_2PO_3;

R^1, R^2, and R^3 are the same or different and are selected from H, C_1-C_6 alkyl, Ph, $PhCH_2$-; and

Y represents (p-C_6H_4)n where n=0-2.

42. (New) The method of claim 35, wherein the modified biological molecule is of formula (III)

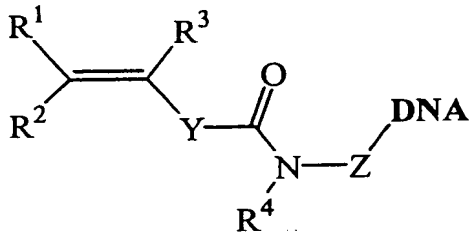


(III)

wherein: DNA represents a DNA fragment; R^1, R^2, and R^3 are the same or different and are selected from H, alkyl C_1-C_6, Ph, $PhCH_2$-; and

Y is $(p\text{-}C_6H_4)_n$ where n=0-2.

43. (new) The method of claim 35 wherein the modified biological molecule is of formula (IV)



IV

wherein:

DNA represents a DNA fragment;

R^1, R^2, and R^3 are the same or different and are selected from H, alkyl C_1-C_6, Ph, $PhCH_2$-; and

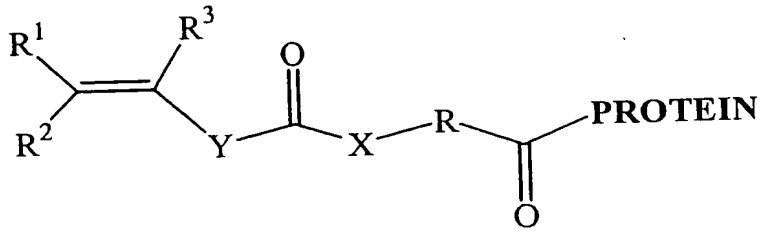
Y is $(p\text{-}C_6H_4)_n$ where n=0-2;

R_4 represents H, $(CH_2)_nOH$ where n=2-6; and

Z is $(CH_2)_nCH(CH_2OH)CH_2OX$ where n=1-6; or $(CH_2)_n$-OX where n=2-6;

X is a phosphodiester group binding an unsaturated moiety to 5'- end, 3'-end or both of the DNA fragment..

44. (new) The method of claim 35 wherein the modified biological macromolecule is a protein of formula (V)



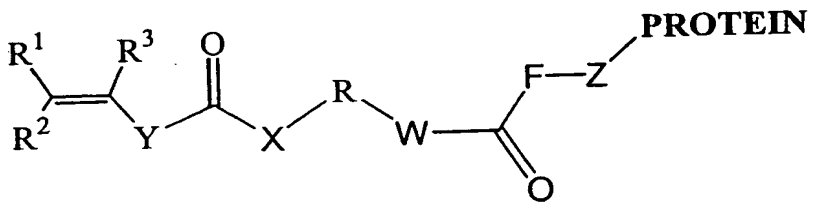
V

wherein R^1, R^2, and R^3 are the same or different and are selected from H, alkyl C_1-C_6, Ph, $PhCH_2$-;
X is NH, O, CH_2, or S;

Y is $(p\text{-}C_6H_4)_n$ where n=0-2 and

R is $(CH_2)_n$, $(CH_2CH_2O)_n$ where n=1-20.

45. (new) The method of claim 35 wherein the modified biological macromolecule is a protein of formula (VI)



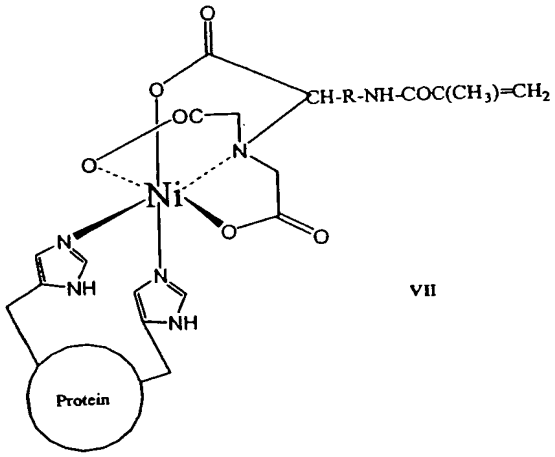
VI

wherein R^1, R^2, and R^3 are the same or different and are selected from H, alkyl C_1-C_6, Ph, $PhCH_2$-;

X is NH, O, CH_2, or S;
Y is $(p\text{-}C_6H_4)_n$ where n=0-2; and

R is $(CH_2)_n$, $(CH_2CH_2O)_n$ where n=1-20.

46. (new) The method of claim 35 wherein the modified biological macromolecule is a protein of formula (VII)



VII

wherein R is $(CH_2)_n$, $(CH_2CH_2O)_n$ where n=1-20.

47. (new). The method of claim 35 wherein use is made of a water soluble high-boiling organic compound as a component (D) of medium for performing a copolymerization.

48 . (new) The method of claim 47 wherein use is made of N,N-dimethylformamide and dimethylsulfoxide as a water soluble high-boiling organic compound.

49. (new) The method of claim 35 wherein use is made of a water soluble polyhydric compound as a component of medium for performing photo initiated polymerization.

50. (new) The method of claim 49, wherein the one or more water soluble polyhydric compound is selected from glycerol, sucrose and polyvinyl alcohol.

51. A method for preparing a composition (K) according to claim 35 for immobilization of biological macromolecules in hydrogels by using a copolymerization consisting in that components of mixture of the following formula:

K=aA+bB+cC+dD+eE

wherein:

A is a monomer based on derivatives of acrylic and methacrylic acids;

B is a water soluble cross-linking agent;

C is a biological modified macromolecule bearing an unsaturated group;

D is a water soluble compound as a medium component for performing a copolymerization;

E is water, and

a, b, c, d, and e are percentages (X) of each ingredient in the composition, wherein for solids X=m/v x 100% and for liquids X= v/v x100%, wherein the total content of monomer and cross-linking agent ranges from 3 to 40% ($3 \leq (a + b) \leq 40\%$), and a monomer to cross-linking agent ratio being within a range of 97:3 to 60:40 and percentages of C, D, and E ingredients being within a range of $0.0001\% \leq c \leq 10\%$; $0\% \leq d \leq 90\%$; $5\% \leq e \leq 95\%$ are mixed until the formation of a homogeneous solution which being degassed and being useful for production of biochips.